

# BI-LEVEL IMAGE THRESHOLDING

## *A Fast Method*

António dos Anjos and Hamid Reza Shahbazkia

*Faculty of Sciences and Technology, University of Algarve, DEEI - ILab 2.57, 8005-139 Faro, Portugal*

**Keywords:** Bioinformatics, Medical image processing, Image thresholding.

**Abstract:** Images with two dominant intensity levels are easily manually thresholded. For automatic image thresholding, most of the effective techniques are either too complex or too eager of computer resources. In this paper we present an iterative method for image thresholding that is simple, fast, effective and that requires minimal computer processing power. Images of micro and macroarray of genes have characteristics that allow the use of the presented method for thresholding.

## 1 INTRODUCTION

It is known that, in the context of image processing, thresholding (Sezgin and Sankur, 2004) is a simple, but powerful tool to separate objects from the background. There is a vast number of applications for thresholding such as document image analysis (Kamel and Zhao, 1993), map processing (cad, ), scene processing and quality inspection of materials (Sezgin and Tasaltin, 2000). Gene images (Diachenko, 1996)(Zhang, 1999) of micro and macro-arrays, where dots of cDNA need to be extracted from the background and electrophoresis and two-dimensional electrophoresis (Dowsey and Yang, 2003) gels, where blots need to be extracted from the background, to determine protein expression, are more recent applications for image thresholding. The quality of the subsequent steps (e.g. spot detection, quantification) will often depend on the quality of the image thresholding.

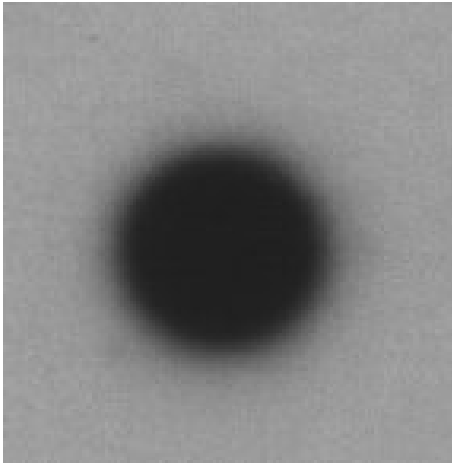
In this paper it is presented a method of image thresholding that aims to be simple – allowing a rapid implementation in any computer programming language, fast – requiring low computing power – and effective – giving results that can be compared with other reference methods of image thresholding. First it will be presented an overview of what lead us to the proposed method, then, the method itself will be described. Finally, there will be presented some results and comparative data with reference methods of thresholding.

## 2 STATISTICAL APPROACH

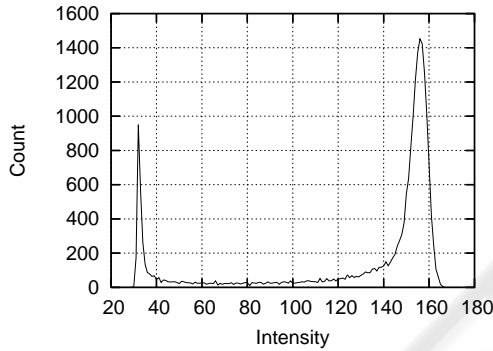
After statistically (Kilian, 2001) analysing several histograms of genomic images with two dominant intensity classes, the background intensity class and the foreground intensity class, with one of them being dominant over the other, for example, the background class being dominant over the foreground class (see fig. 1), it was noticed that when the histogram grows substantially near the dominant peak, the variance, from the lowest intensity level to the intensity level where the big growth happens, decreases relatively to the variance from the lowest intensity level to that intensity level minus one.

In the case of figure 1, the decrease on variance, obviously, happens because the background is highly dominant. From the intensity level where that change occurred to a very fair threshold level there is a distance of about minus one standard deviation of the whole image histogram. With images with a great contrast between background and foreground, the standard deviation is bigger, and the contrary also happens. Thus, the goal was to find where the decrease of variation occurred and then subtract one standard deviation of distance. Very good results were achieved with images where the background was the dominant class but, as long there is one dominant class, it doesn't matter which is the dominant one.

If the variance is measured starting on the first intensity level to any level before the least dominant peak, a decrease on the variance may happen before reaching the most dominant one. Ideally, the



(a) Macroarray dot.



(b) Histogram of 1(a).

Figure 1: Two-dominant intensities image.

decrease should be measured having, as first ending level, the level where we find the depression between the two peaks, but, if that could easily be found, there wouldn't be needed to proceed, because that is what we are trying to find. For that reason, the mean intensity level of the image was used as the first ending level.

Clearly, if the mean is above the point where the variance starts to decrease, which happens if the least dominant class (peak) has very little representation in contrast with the dominant class, the method will not work, even if there is an important contrast between classes. Nevertheless, for images where the mean was below the point of decrease on variation, this solution finds a fairly good threshold level. So, the variance was measured, starting from the first intensity level until the mean intensity, towards the dominant peak, comparing the result of each step with the previous one.

Knowing that an image is a 2D grayscale intensity function with  $N$  pixels with graylevels from 1 to  $L$

and the number of pixels of graylevel  $i$  is denoted by  $f_i$ , we can define the mean until level  $l$  as:

$$\mu_l = \frac{\sum_{i=0}^l i \times f_i}{\sum_{i=0}^l f_i}; \quad (1)$$

and the variance until level  $l$  as:

$$\sigma_l^2 = \frac{1}{\sum_{i=0}^l f_i} \sum_{i=0}^l f_i \times (i - \mu_l)^2. \quad (2)$$

Assuming that the dominant peak is on the right side of the histogram (as in image 1(b)), the level  $l$  where  $\sigma_l^2$  will decrease relatively to  $\sigma_{l-1}^2$ , can be defined as the first occurrence of:

$$l^* = \{l \mid \sigma_l^2 < \sigma_{(l-1)}^2 \wedge \mu_L \leq l < L\}; \quad (3)$$

then, the threshold level  $T$ , would be:

$$T = l^* - \sqrt{\sigma_L^2}. \quad (4)$$

where  $\sqrt{\sigma_L^2}$  is the standard deviation of the whole image.

As an example, take the case of the image  $I$  in figure 1(a). The mean intensity of  $I$  is  $\mu_L = 130$  and the standard deviation is  $\sigma_L = 43$ , with  $L = 256$ . Calculating the variances  $\sigma_l^2$  with  $l$  being the ending levels for calculation of the variance and  $\mu_L \leq l < L$ , all the variances will rise until the gray level is 153 (see fig. 2), where  $\sigma_{154}^2 < \sigma_{153}^2$ . Consequently,  $l^* = 154$ , and the estimated threshold level is calculated by subtracting the standard deviation of the image from  $l^*$ . In this way, we get a threshold value of  $T = 111$  (see fig. 3). Applying the well known Otsu (Otsu, 1979) thresholding method, the achieved threshold level was of 100. Taking in consideration that in many circumstances an acceptable threshold level for this image could be between the intensities 40 and 140, 111 is a good threshold level. For the specific case, any threshold level between 60 and 120, would be a very good threshold level. When manually thresholding the same image, the optimum threshold was found as 76. With  $T = 76$ , the diffuse area that separates background and foreground was almost completely eliminated.

As can be seen on figure 2, the variance decreases when the selected part of the histogram starts to grow more in height than in spread, meaning that all the intensity values until  $l^*$  or higher will be closer to the mean. On a histogram with both intensity classes equally expressed, the growth of any of the existing intensities will decrease the variance. Observing figure 2, it can be stated that the variance decreases after the histogram is more or less balanced between

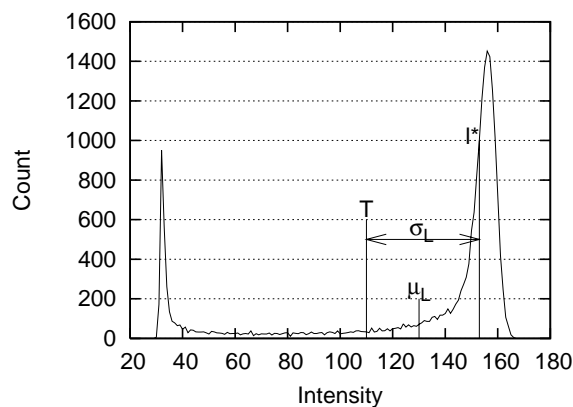


Figure 2: Calculation of T.

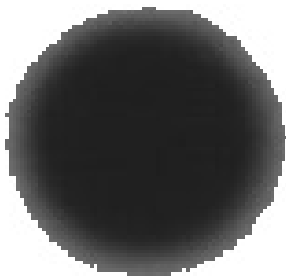


Figure 3: Image 1(a) thresholded with T=110.

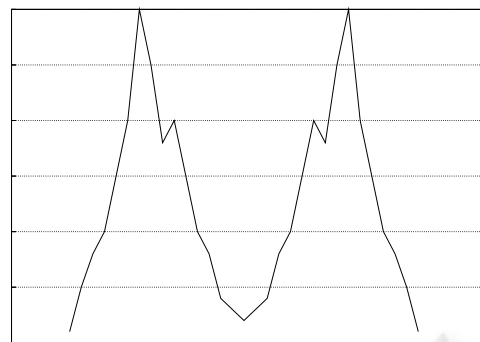
the first intensity level and level  $I^*$ . Also, considering a histogram with only two intensities, the maximum variance will be reached when both intensities are equally expressed. Any increase or decrease to any of the intensities of the histogram, will result in the decrement of the variance, because one of the intensities will be dominant.

As stated before, the previous approach would need images with very specific characteristics and, that is not always possible. Although this was not a good technique for image thresholding it gave us some ideas on how to find a good thresholding level, as explained on the next section.

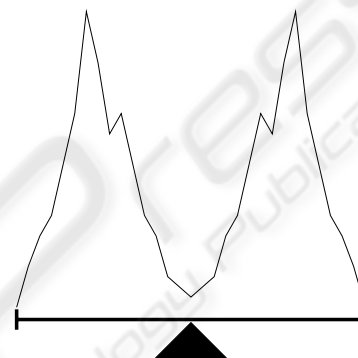
### 3 WEIGHTING A HISTOGRAM

If a perfect balanced histogram, i.e. a histogram that has the same distribution of background and foreground, could be placed over a lever, it wouldn't fall for any side (see fig. 4). Also, notice that the optimum threshold level would be right in the centre of the lever.

Looking at figure 5, which represents an unbalanced histogram, it can be observed that the lever falls



(a) Balanced Histogram.



(b) Histogram over a lever.

Figure 4: Perfectly balanced histogram.

to the side where the histogram is heavier. The idea is to try to balance the unbalanced histogram. Once again, there is the assumption that the image has two dominant classes (peaks), one representing the foreground and the other representing the background. In this case, there is no need of one being highly dominant over the other. So, how can an unbalanced histogram be balanced? The proposed way of doing it is to figuratively put the unbalanced histogram over a lever, as in figure 5, and then start to remove the excess weight from the heavier side. The next step is to adjust the base triangle to the new middle position.

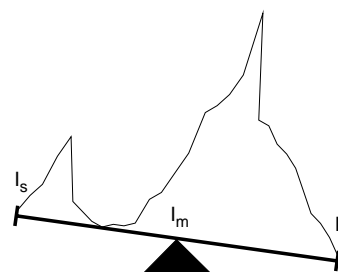


Figure 5: Unbalanced Histogram.



area of the least dominant peak, moving  $I_m$  closer to the least dominant peak. The approach represented on figure 6(b) can correct this problem only if the resulting  $I_m$  is still between the two peaks. In the case that it's the most dominant peak that has the big tail, that may cause the side that should be the lighter one, to be the heavier, not allowing to find the right thresholding level. In order to solve this problem, there have been found two main approaches. One consists of passing a parameter to the algorithm that will work as the least quantity that will be representative and, thus, accounted for the calculation of  $I_s$  and  $I_e$ . The other approach consists of performing a mean smoothing of the image. This may eliminate all the intensities that have almost no representativity.

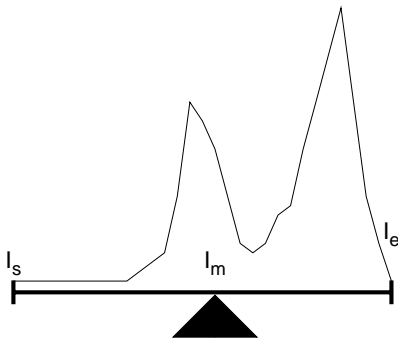


Figure 7: Big tail at the left of least dominant peak.

## 4 PROPOSED METHOD

The method presented in the previous section (see alg. 3.1) has a caveat that makes it unreliable in some cases. This algorithm will tend to approach  $I_m$  to the lowest peak, proportionally to the degree of growing and height of the highest peak. That happens, of course, because each bar of the highest peak will correspond to more than one of the lowest peak bars. This may be a minor problem if the contrast is very high, but will be noticed in low contrast images with one of the peaks much higher than the other and with high accentuation of growth. To solve this problem it was applied the same reasoning presented in section 3. For example, if the right side of the histogram is the heavier, we will remove bars from the right side of the histogram. The stop condition for algorithm 3.1 is when the right side gets lighter than the left side. At this point, the histogram will have its left side heavier than the right side and, thus, it's unbalanced. Applying the same algorithm (mirrored) to the histogram, the right side will become the heaviest side again, and so on. The result is that  $I_s$  and  $I_e$  will get closer until

they are equal to  $I_m$ . This approach is represented by algorithm 4.1.

### Algorithm

#### 4.1: GET-THRESHOLD-FINAL( $f, I_s, I_e$ )

```

 $I_m \leftarrow \frac{I_s + I_e}{2}$ 
 $W_l \leftarrow \sum_{i=I_s}^{I_m} f_i$ 
 $W_r \leftarrow \sum_{i=I_{m+1}}^{I_e} f_i$ 
while  $I_s \neq I_e$ 
    if  $W_r > W_l$ 
        then
             $W_r \leftarrow W_r - f_{I_e}$ 
             $I_e \leftarrow I_e - 1$ 
            if  $\frac{I_s + I_e}{2} < I_m$ 
                then
                     $W_l \leftarrow W_l - f_{I_m}$ 
                     $W_r \leftarrow W_r + f_{I_m}$ 
                     $I_m \leftarrow I_m - 1$ 
            else if  $W_l \geq W_r$ 
                then
                     $W_l \leftarrow W_l + f_{I_s}$ 
                     $I_s \leftarrow I_s + 1$ 
                    if  $\frac{I_s + I_e}{2} > I_m$ 
                        then
                             $W_l \leftarrow W_l + f_{I_{m+1}}$ 
                             $W_r \leftarrow W_r - f_{I_{m+1}}$ 
                             $I_m \leftarrow I_m + 1$ 
    do
return ( $I_m$ )
    
```

In this way  $I_m$  will tend to move to the lowest area of the depression in the histogram. With this approach it won't matter how fast the highest peak grows because  $I_m$  will be centred, once again, if  $I_m$ 's initial position is located in between the two peaks. There is still the concern of the big tails in the histogram, if  $I_m$ 's initial position is outside of the depression in the histogram.

As a final matter, there is the situation when there is very low contrast between background and foreground. In this case we only have a peak. This will result on a all or nothing threshold, because the histogram will always be heavier from the same side.  $I_s$  will still get to be equal to  $I_e$ , but the threshold level may not be correct. When the histogram has two peaks, bars will be removed from both sides of the histogram, so, the solution that was found to this problem was to place a flag in each of the places where bars removal occurs. This way we know if it's a one or two peaks histogram or if it's a one peak histogram only. In the case of a one peak histogram, a good approximation for the threshold level may be the mean of the original histogram minus some constant or some percentage.



## 5 RESULTS

For test and comparison of the presented method, there were used scanned radioactive images of gene macro arrays. Due to the existence of gradient in the global image, obviously there could not be used an optimum global threshold level, so the image was split in to tiles of spot images (see fig. 8).

Table 1, presents the result of applying this algorithm to those images and compares it to the result of applying various reference methods. Results are presented in the following order: Manual threshold, Otsu's, IsoData, Maximum Entropy, Mixture Modelling and the proposed method's threshold levels. For the IsoData, Maximum Entropy and Mixture Modelling threshold methods, it was used ImageJ's (Rasband, 2006) implementation.

Table 1: Results of the various thresholding methods.

Spot	Man	Otsu	Iso	Max	Mix	Pro
1	66	80	78	101	113	69
2	60	68	66	89	12	50
3	59	66	64	85	13	55
4	58	83	81	101	118	67
5	80	90	88	107	118	77
6	55	68	66	89	12	54
7	92	92	90	105	116	84
8	57	69	67	94	104	50
9	60	73	71	96	111	59
10	65	78	77	96	112	72

As can be seen in table 1, the proposed method is the one that, generally, finds lower values for thresholding. As the spot starts to blend with the foreground (the diffuse area) there will be the depression in the histogram. That diffuse area increases in intensity level and in quantity of pixels as it goes from the spot to the foreground. It is normal, then, that the proposed method will find the lower threshold levels because, as stated before,  $I_m$  will tend to move to the lowest area of the depression of the histogram. Figure 8 is shown just to present an idea of the criteria that was used in the manual threshold calculation (the ground truths).

## 6 CONCLUSIONS

This is a very fast method that works very well on images that have a fair amount of background and foreground representation, and with a reasonable contrast between both, as is the case of scanned radioactive images of macroarrays. It is a good approach when

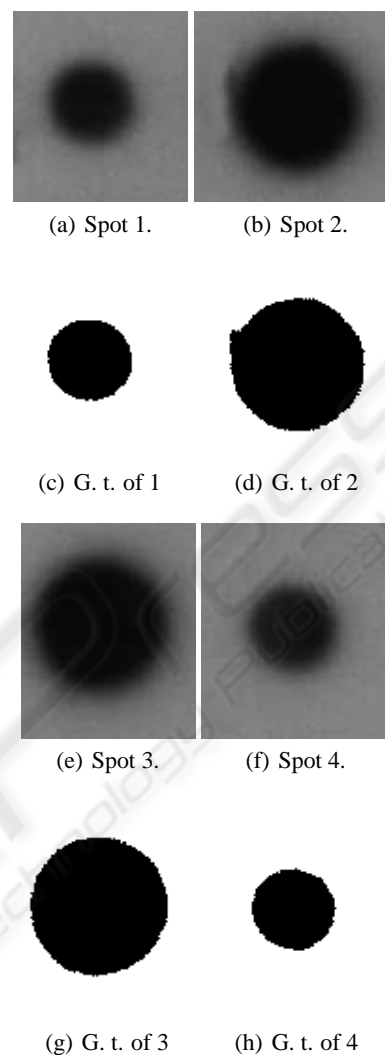


Figure 8: First four of the tested sample images and their ground truths.

the objective is to find the spot without the diffuse area. If the diffuse area is to be included, Otsu or Iso-data may be better. The presented method is already being used on a bioinformatics software (Anjos and Ascenso, 2007) for analysis of gene expression data of macroarray images.

## REFERENCES

- Anjos, A., S. H. and Ascenso, R. (2007). Maq - a bioinformatics tool for macroarray analysis. (*Internal Report - ILAB - UA1g*).
- Diachenko, L., e. a. (1996). Suppression subtractive hybridization: A method for generating differentially

- regulated or tissue-specific cdna probes and libraries. *Proc. Natl. Acad. Sci. USA*, 93(12):6025–6030.
- Dowsey, Andrew, D. M. and Yang, G.-Z. (2003). The role of bioinformatics in two-dimensional gel electrophoresis. *Proteomics*, 3(8):1567–1596.
- Kamel, M. and Zhao, A. (1993). Extraction of binary character/graphics images from grayscale document images. *Graphical Model and Image Processing*, 55(3):203–217.
- Kilian, J. (2001). Simple image analysis by moments. *OpenCV library documentation*.
- Otsu, N. (1979). A threshold selection method from gray-level histograms. *IEEE Trans. Systems Man Cybernet*, 9:62–69.
- Rasband, W. (1997-2006). Imagej. *U. S. National Institutes of Health, Bethesda, Maryland, USA*, page <http://rsb.info.nih.gov/ij/>.
- Sezgin, M. and Sankur, B. (2004). Survey over image thresholding techniques and quantitative performance evaluation. *Journal of Electronic Imaging*, 13:146–165.
- Sezgin, M. and Tasaltin, R. (2000). A new dichotomisation technique to multilevel thresholding devoted to inspection applications. *Pattern Recognition Letters*, 21:151–161.
- Zhang, M. (1999). Large-scale gene expression data analysis: a new challenge to computational biologists. *Genome Research*, 9:681–688.

